*Approach to Atomic Force Microscopy*. Appl. Phys. Lett. 53, 1045-1047 (1988), incorporated herein by reference.

**[0044]** In addition to the aforementioned centering of the tip in the laser beam based on the quadrant photodiode x and y signals, the tip can be centered on the sum signal alone, as illustrated in FIG. **4**. This would allow registered tip exchange based on a simpler optics perhaps with slightly decreased ultimate precision. In FIG. **4**, the tip is held approximately 60 nm above the surface and slowly dithered sequentially in x and y, as depicted schematically in (a). In (b), the sum signal (or total light falling on the quadrant photodiode) is shown (green solid line). The lateral position of the tip is then adjusted to extremize this signal. The same process works in the vertical axis as well. Similarly, in (c), the sum signal is shown (green solid line) as the tip is dithered in y. The tip is centered to the beam in x and y by maximizing the sum signal in each dimension. It is possible to use different algorithms on different tips. Again, any offset can be predetermined and removed.

**[0045]** There is some optical crosstalk between the detection axes that leads to an erroneous signal on one axis when moving another. Substantial crosstalk (approximately 20%) is often observed when calibrating the atomic force microscope tip, because it is a geometrically asymmetric object tilted at 15° with respect to the y-axis (the cantilever's long axis). Field-programmable gate array (FPGA) cards (PCI-7833R and PCI-7831R, National Instruments) can be used to provide the computational power to parameterize and minimize such crosstalk via linear algebra-based algorithms, as described in Lang, M. J., Asbury, C. L., Shaevitz, J. W. & Block, S. M. *An automated two-dimensional optical force clamp for single molecule studies*. Biophys. J. 83, 491-501 (2002) and Churnside, A. B., King, G. M., Carter, A. R. & Perkins, T. T. *Improved performance of an ultrastable measurement platform using a field-programmable gate array for real-time deterministic control*. Proc. of SPIE 7042, 704205 (2008), both incorporated herein by reference in their entirety. FPGAs also provide for more rapid (500 Hz) and therefore precise controlling of all six axes of motion, leading to tip control of <0.04 nm in three dimensions (3D) in air.

**[0046]** For absolute alignment, all fiducial marks are scanned. This process allows the absolute center of each mark to be established and aligned by repositioning the precision translation stage. The feedback loop then maintains the alignment stably.

**[0047]** It should be understood that at least one of the two or more structures is preferably mounted on a precision positioning structure, referred to generically as a precision positioner. The precision positioner can be piezoelectric, and can include, but is not limited to, a translational stage. Preferably, the precision positioner comprises a nanopositioning system and the distance separating the two or more structures comprises a picometer-scale precision for a period of time or time interval. The precision positioner can comprise scanning stages and a high speed, multi-axis nanopositioning system. The translational stage, which is the preferred positioner, can be a two dimensional or three dimensional stage. One preferred embodiment is a closed loop, direct drive, 3D piezoelectric transducer (PZT) stage, such as P363.3CD and P733.3DD, Physik Instrumente.

**[0048]** The present invention utilizes a feedback loop. The related characteristic of the signal used for feedback purposes can be proportional, differential gain, integral gain, or any other mathematical relationship that can be used to provide a feedback loop.

**[0049]** Alternatively, a comparator could be used instead of a feedback algorithm or processor to compare the signal output from the photosensitive device with a control signal corresponding to a constant amount of separation between the structures. A person of ordinary skill in the art appreciates that the differential or comparison criteria could be any of voltage, current, power, phase, or frequency.

**[0050]** The sample is preferably associated with a substrate. Exemplary substrates can include glass cover slips or opaque substrates. The sample may also be presented in alternative environments, such as vacuum, water or other liquid, and air. Different substrates and being in air and water yields similar results. FIG. **3** illustrates (a) Quadrant photodiode (QPD) signals as the tip was scanned along the x-axis through the detection laser. These records were measured through microscope cover glass with the tip in air (black) and submerged in water (grey).

**[0051]** Metallic or semiconducting substrates are compatible with the proposed optical stabilization. FIG. **1** shows two different detection geometries. In FIG. **1**A, the detection laser is directly scattered off of the apex of the probe tip. Using this geometry with unmodified commercial tips and several substrates (glass, mica, thin metal films), the present method has been used to demonstrate tip detection in air and fluid at room temperature. The shape and material properties of the tip, as well as the wavelength of the laser, can be varied to suit different applications. For example, silicon wafers can be accommodated by using laser wavelengths where silicon is transparent.

**[0052]** Opaque or highly scattering substrates utilize a straightforward modification of detection geometry. Detection would come from above and a fiducial mark would be engineered near the base of the cantilever, identical to the sample's fiducial mark (FIG. **1**B). A further benefit of this geometry is the ability to detect and compensate for noise in the z position of the tip holder. Such active noise suppression leaves only height changes on the sample to be measured by cantilever deflection. This six-axis active stabilization would be immediately applicable to industrial applications where, for example, AFMs are commonly operated in close proximity to noise generating semiconductor process equipment.

**[0053]** The present invention utilizes commonly available, commercial, unmodified tips. The useful tips can be of any size and shape, for example, symmetric pyramidal shaped tips. The technique works with uncoated as well as metalized tips. Exemplary tips include, but are not limited to scanning tunneling microscope tips, atomic force microscope tips, near field scanning optical microscope tips, pipette tips, etc. The tip can act as its own fiducial or a fiducial mark can be engineered elsewhere on, near or into the cantilever assembly.

**[0054]** This process can be repeated with different types of tips. For tips whose structure was highly asymmetric (e.g. Olympus biolevers), a large offset may be found in the one axes, but a small offset in the other (e.g. [0 nm, −1000 nm]. This offset is generally reproducible between tips with the same class. This non-time varying offset can be subtracted once characterized. For improved registration of highly asymmetric tips, it is advantageous to align the tip and the stage in three dimensions.

**[0055]** Alignment can be automated based on the tip signal. Different alignment algorithms may yield slightly different